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Free Amino Acids Production by Ectomycorrhizal Fungi of Pine (*Pinus sylvestris* L.)

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Abstract

Studies on free amino acids production by five species of ectomycorrhizal fungi (*Amanita muscaria*, *Suillus granulatus*, *Suillus luteus*, *Suillus bovinus* and *Rhizopogon luteolus*) show that all the fungi produced mainly: glutamic acid, leucine, lysine, ornithine, arginine and an unidentified ninhydrin-positive compound X_3. Both the quality and quantity of amino acids released was different in the fungal species studied. The predominant amino acids in post-culture liquids in general did not exceed 1.5 μg mg dry mass.

Introduction

Amino acids and plant growth regulators are considered to be of importance in the formation and function of mycorrhizae in forest trees (Melin, 1953; Norkrans, 1953; Moser, 1959; Krupa, Fontana and Palenzona, 1973; Krupa and Bränström, 1974; Peller and Mullins, 1982).

Studies on free and bound amino acids production by the ectomycorrhizal fungus *Pisolithus tinctorius* were performed by Booker (1980) and Peller and Mullins (1982). The content of such amino acids in non-mycorrhizal and mycorrhizal roots of pine has been determined by Krupa, Fontana and Palenzona (1973), Krupa and Bränström (1974). Little however is known about the excretion of free amino acids by ectomycorrhizal fungi into the environment (France and Reid, 1983). Therefore the aim of our work was to study the qualitative-quantitative production of free amino acids by five ectomycorrhizal fungi of pine.

Experimental

Materials and Methods

Five strains of ectomycorrhizal fungi: *Amanita muscaria* (L. ex Fr.) O. Kuntze, *Suillus granulatus* (L. ex Fr.) O. Kuntze, *Suillus luteus* (L. ex Fr.), S. F. Gray, *Suillus bovinus* (L. ex Fr.) O. Kuntze and *Rhizopogon luteolus* Fr. and Nordh. were used in our studies.

The fungi were grown in 1 l Erlenmeyer flasks containing 100 cm^3 of a modified liquid Lamb's (1974) medium (glucose, 10.0 g; NH_4Cl, 500 mg; $MgSO_4 \times 7H_2O$, 50 mg; KH_2PO_4, 50 mg; FeEDTA, 1 cm^3 of 5 ppm Fe solution; thiamine-HCl, 1.0 mg; biotin 0.01 mg; distilled water, 1 l; pH 5.5—5.7) for 21 days at 26°C. Each flask was inoculated with three discs (1 cm diameter cut from the edge three week-old agar culture).

After incubation the mycelium was separated by filtration on filter paper and the dry mass was determined. The supernatant was passed through a Dowex 50W X8 cation exchange column (H^+ form, 100—200 mesh) and eluated with concentrated ammonia. The amino acids contained in such eluates were separated by paper electrophoresis (acidic and basic) and paper electrochromatography (neutral). Amino acids on the electrochromatograms and electrophoregrams were detected by spraying with ninhydrin reagent and heating at 85°C for 15 min. Details concerning the methods applied are given in a previous paper (Różycki and Strzelczyk, 1985).

In qualitative determinations of the amino acids the size and colour intensity of the spots after dipping in ninhydrin reagent were considered. On the basis of different numerical values (0—4) considering different classes of spot size and colour intensity, index of total production of the amino acids was calculated (arithmetical mean of numerical values concerning all amino acids produced by one strain). Similar method was applied by Ruiz-Berraquero and Ramos-Cormenzana (1977). However in their studies the basis for calculation of the above index was the growth intensity of auxotrophic bacterial strain around bacterial colony excreting the amino acid into the agar medium.

Quantitative determinations of the amino acids (glutamic and aspartic acids, alanine, leucine, lysine, arginine and ornithine) were carried out by measuring the extinction (504 nm) of methanolic eluates of the spots, after the reaction with Barrolier's cadmium-ninhydrin reagent (Clotten and Clotten, 1962).

Results

The results of our studies are presented in Tables I—II and on Fig. 1. All the fungi studied released 22 different ninhydrin-positive compounds. Glutamic acid, alanine, leucine, basic amino acids (lysine, ornithine, arginine) and unidentified ninhydrin-positive compound X_3 were produced most frequently and in greatest amounts.

A. muscaria produced different most actively amino acids. Lysine, alanine, leucine and glutamic acid were predominant among the amino acids produced by this fungus. *S. granulatus* was a weak amino acid producer *S. luteus* synthesized leucine and trace amounts of other amino acids (glutamic acid, glycine, alanine, valine, arginine and X_3) — Table I.

In post-culture liquids of *S. bovinus* and *R. luteolus* basic amino acids (*S. bovinus* — ornithine and arginine; *R. luteolus* — lysine, ornithine, arginine and histidine) were predominating. *R. luteolus* excreted also relatively large amounts of two unidentified ninhydrin-positive compounds: X_3 and X_{Arg}.

Table II presents indices of the total productive of the amino acids. These indices are in the following order: *A. muscaria* > *R. luteolus* ≥ > *S. bovinus* > *S. granulatus* > *S. luteus*.

Table 1

Free amino acids production by ectomycorrhizal fungi

Fungal species	Amino acids*																				
	Acidic		Neutral												Basic				Unidentified		
	Glu	Asp	Gly	Ala	Leu	Val	Phe	Ser	Thr	Asp-NH_2	Tyr	Met	Pro	Cys	β-Ala	Lys	Orn	Arg	His	X_3	X_5	X_{Arg}
A. muscaria	3	2	3	4	4	3	2	2	3	1	2	1	2	1	0	4	2	2	3	3	2	1
S. granulatus	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	2	1	2
S. luteus	1	0	1	1	3	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1
S. bovinus	1	1	1	1	3	1	0	1	0	0	0	0	0	0	0	0	3	2	0	1	0	1
R. luteolus	1	1	1	1	0	0	0	0	0	0	1	0	0	0	1	3	2	3	2	3	1	3

* 4 — amino acid in large amounts (large strong coloured spots); 3 — amino acid in considerable amounts (distinct spots); 2 — amino acid in small amounts (small, weak coloured spots); 1 — traces of amino acid; 0 — amino acid not detected

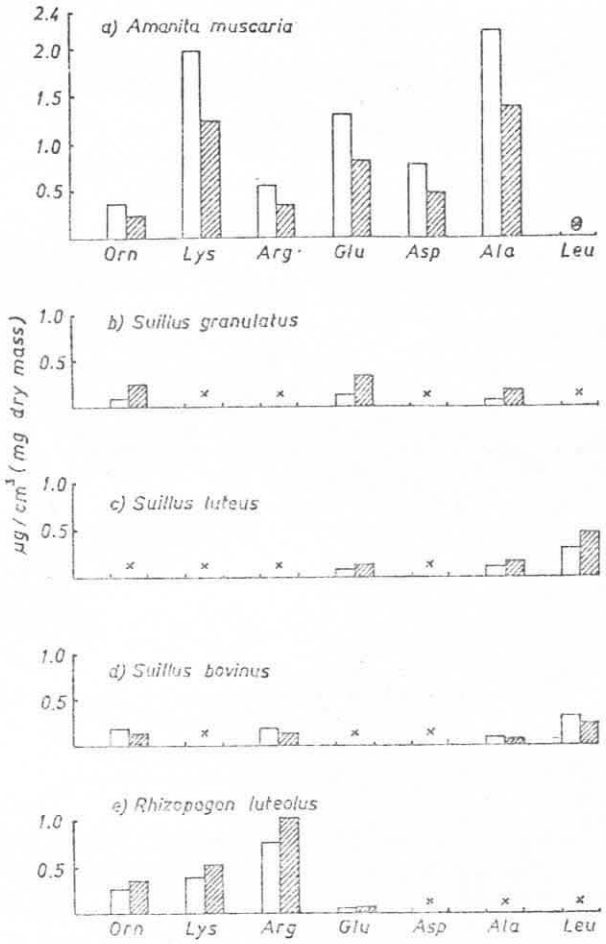

Fig. 1. Amounts of amino acids produced by ectomycorrhizal fungi.

Empty histograms – µg/cm³, cross hatched histograms – µg/mg dry mass, x – not determined because of trace amount (except leucine Ø which could not be separated from other amino acids on the electrophoregram).

Table II

Index of total production of the amino acids by ectomycorrhizal fungi

Fungal species	Index*
A. muscaria	2.27
S. granulatus	0.54
S. luteus	0.45
S. bovinus	0.82
R. luteolus	1.04

Fig. 1 presents the results of quantitative determinations of some amino acids. In the culture filtrate of *A. muscaria* approximately 1.5 μg of lysine and alanine per 1 mg of dry mass were found; the remaining cultures produced less of these amino acids (Fig. 1).

Discussion

It appears from the results obtained in our work that the ectomycorrhizal fungi studied excrete the same amino acids as do other soil and root-zone microorganisms (Perlman and O'Brien, 1958; Vágnerová and Vančura, 1962; Schinner, 1976; Ruiz-Berraquero and Ramos-Cormenzana, 1977; Chatterjee and Chatterjee, 1982; Różycki and Strzelczyk, 1983). *A. muscaria*, *S. bovinus* and *R. luteolus* synthesized more basic amino acids than the bacteria (Różycki and Strzelczyk, 1985). However glutamic acid was produced by bacteria in amounts several times higher than by mycorrhizal fungi (Różycki and Strzelczyk, 1985).

Amino acids are of importance for the host-plant and fungus interrelationship (France and Reid, 1983). Krupa, Fontana and Palenzona (1973) and Krupa and Bränström (1974) found no sharp differences in quantitative-qualitative composition of free and bound amino acids in mycorrhizal and non-mycorrhizal roots of pine. According to the above authors in consequence of the limitation of own arginine biosynthesis, ectomycorrhizal fungi may most likely metabolize the host's pool of arginine providing thus the root with other amino acids — conversion products of arginine (glutamic, aspartic and γ-aminobutyric acids, glutamine, asparagine, ornithine and glycine). Some ectomycorrhizal fungi like *Suillus variegatus* and *P. tinctorius* may synthesize ornithine cycle metabolites like ornithine and arginine, detected in the free amino acids pool of the mycelium (Krupa and Bränström, 1974; Booker, 1980). In our studies the above amino acids were detected in the culture filtrates of *A. muscaria*, *S. bovinus* and *R. luteolus*.

Booker (1980) observed that the main component of bound amino acids in the mycelium of *P. tinctorius* was glutamic acid, but among free amino acids alanine predominated. She supposes that the two above compounds are the primary products of the amino acids synthesis from ammonium ions and α-ketoacids. This fungus is also able to utilize ammonium ions for biosynthesis of glutamic acid or glutamine in symbiosis with *Pinus contorta* (France and Reid, 1983). These authors have shown the possibility of translocation in and utilization by the plant of the amino acids released by the fungal symbiont. It could be equally advantageous for the host-plant, as well as for providing sugars and vitamins for the fungus (Haeskaylo, 1971). In our work substantial amounts of glutamic acid and alanine were found only in the post-culture liquids of *A. muscaria*

It can be supposed that in natural conditions ectomycorrhizal fungi release amino acids not only into the plant tissues, but also into the soil. These compounds and other fungal metabolites as well as the root exudates passing through the fungal mantle affect the development of mycorrhizosphere microorganisms (Katznelson, Rouatt and Peterson, 1962; Neal, Bollen and Zak, 1964; Rambelli, 1973; Różycki, Dahm, Strzelczyk, Prusinkiewicz and Kowalski, 1983).

Many root zone microorganisms were found to require the sulphur containing amino acids for their optimum development. (Wallace and Lochhead, 1950; Vágnerová and Vančura, 1962). The mycorrhizal fungi studied in this work did not release sulphur containing amino acids (only *A. muscaria* excreted trace amounts of cysteine and methionine).

The fungal symbionts of forest trees are also affected by egzogenous amino acids. Amino acids are excreted by the roots and microorganisms of the mycorrhizosphere (Slankis, Runeckles and Krotkov, 1964; Schinner, 1976; Różycki and Strzelczyk, 1985). Amino acids (especially glutamic and aspartic acids) stimulate the mycelial growth of some ectomycorrhizal fungi e.g. *Cenococcum graniforme* and various species of the genus *Tricholoma* (Melin, 1953; Norkrans, 1953). Peller and Mullins (1982) noted a stimulative effect of glutamic acid and casein hydrolysate on *P. tinctorius* with a simultaneous increase of threonine and serine in the free amino acids pool of the mycelium.

Exogenous amino acids affect also production of plant growth hormones (mainly auxins) by mycorrhizal fungi. Amino acids containing large amounts of nitrogen (alanine, asparagine, glycine, glutamic acid) are known to be inhibitors of biosynthesis of auxins. On the other hand those containing small amounts of this element (tryptophane — auxin precursor) stimulate the synthesis of auxins by fungi and enhance mycorrhizae formation (Moser, 1959; Slankis, 1973; Rudawska, 1982). Fries (1976) observed a stimulative effect of glutamic acid and casein hydrolysate on basidiospore germination of *S. luteus*.

Harley (1948) pointed out that saprophytic soil microorganisms may affect plant growth and the mycorrhiza formation by the metabolites they excrete (among them also amino acids). On the other hand the metabolites of ectomycorrhizal fungi, may affect the mycorrhizosphere microorganisms (Moser, 1959; Rambelli, 1973; Slankis, 1973). For a better understanding of the complex mechanisms of plant — mycorrhizal fungi and mycorrhizosphere microorganisms interactions further studies on organic substances production by these organisms are required.

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